Romaine lettuce latex deters feeding of banded cucumber beetle: a vehicle for deployment of biochemical defenses

Amit Sethi[1], Heather J. McAuslane[1]*, Hans T. Alborn[2], Russell T. Nagata[3] & Gregg S. Nuessly[3]

[1]Department of Entomology and Nematology, PO Box 110620, University of Florida, Gainesville, FL 32611-0620, USA, [2]Center for Medical, Agricultural, and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, FL 32608, USA, and [3]Everglades Research and Education Center, University of Florida, 3200 East Palm Beach Road, Belle Glade, FL 33430-4702, USA

Abstract

Latex is widely found among plant species and is known to play a defensive role against certain herbivores. Two romaine lettuce, *Lactuca sativa* L. (Asteraceae) cultivars, 'Valmaine' (resistant) and 'Tall Guzmaine' (susceptible), were selected to study the potential of latex as a defense mechanism against the banded cucumber beetle, *Diabrotica balteata* LeConte (Coleoptera: Chrysomelidae). Latex from Valmaine strongly inhibited feeding of adult *D. balteata* compared to latex from Tall Guzmaine when applied to the surface of artificial diet. Beetles consumed significantly less diet from disks treated with Valmaine latex than they consumed from diet treated with Tall Guzmaine latex, in both choice and no-choice tests. In a choice test involving diet disks treated with Valmaine latex from young leaves vs. disks treated with latex from mature leaves, the beetles consumed significantly less diet treated with latex from young leaves. No significant difference in diet consumption was found between diets treated with latex from young and mature leaves of Tall Guzmaine in choice tests. Three solvents of differing polarity (water, methanol, and methylene chloride) were tested to extract deterrent compounds from latex; Valmaine latex extracted with water:methanol (20:80) strongly inhibited beetle feeding when applied to the surface of artificial diet. In no-choice tests, fewer beetles were observed feeding on diet treated with water:methanol (20:80) extract of Valmaine latex than on diet treated with a similar extract of Tall Guzmaine latex, resulting in significantly less consumption of the diet treated with the Valmaine latex extract. These studies suggest that moderately polar chemicals within latex may account for resistance in Valmaine to *D. balteata*.

Introduction

Latex is the common term used to describe a frequently milky plant exudate that is typically stored under positive pressure within specialized vessels called laticifers. These laticifers accompany the vascular bundles and ramify into the mesophyll to reach the epidermis (Hayward, 1938; Esau, 1965; Olson et al., 1969; Metcalfe & Chalk, 1983; Fahn, 1990; Kekwick, 2001). Between 12 500 and 20 000

*Correspondence: Heather J. McAuslane, Department of Entomology and Nematology, PO Box 110620, University of Florida, Gainesville, FL 32611-0620, USA. E-mail: hjmca@ufl.edu

plant species, belonging to >900 genera from about 40 families, most of which are dicotyledons, are known to exude latex (Esau, 1965; Lewinsohn, 1991; Kekwick, 2001; Evert, 2006). Latex may contribute to plant defense in two different ways, through either its physical properties (i.e., stickiness) and/or chemical properties (i.e., toxic constituents). Stickiness can result in the entrapment or gumming up of the mouthparts of herbivorous insects (Dillon et al., 1983; Dussourd, 1993, 1995; Zalucki & Malcolm, 1999). Latex contains toxic constituents including alkaloids (Roberts, 1987; Valle et al., 1987; Konno et al., 2006), cardiac glycosides (Zalucki & Brower, 1992; Zalucki & Malcolm, 1999), and terpenoids (Evans & Schmidt,

1976; Rees & Harborne, 1985; Spilatro & Mahlberg, 1986). Some insects circumvent the mechanical stickiness and toxic effects of latex by severing laticifers or by cutting trenches prior to consuming the distal tissue (Dussourd, 1993; Zalucki & Malcolm, 1999; Sethi et al., 2006).

Lettuce, *Lactuca sativa* L. (Asteraceae), is one of the most important vegetable crops grown throughout the world and its production increases annually (USDA, 2005). As a cultivated crop, lettuce is vulnerable to attack by various insect pests including the banded cucumber beetle, *Diabrotica balteata* LeConte (Coleoptera: Chrysomelidae) (Nuessly & Nagata, 1993). In southern Florida, foliar feeding on lettuce by *D. balteata* adults leads to economic damage due to reduction in market grade standard, decreased photosynthetic area, frass contamination of the heads, and increased vulnerability to diseases. Growers are currently dependent on pesticides (Nuessly & Nagata, 1993), which can pose a threat to the environment, farm workers, and natural enemies of insect pests, and increase production costs.

Host plant resistance was explored as an alternative strategy for the management of this economic insect pest in a cos (or romaine) lettuce cultivar, 'Valmaine'. A high level of resistance was reported in Valmaine, compared to the closely related susceptible cultivar 'Tall Guzmaine', against serpentine leafminer, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) (Nuessly & Nagata, 1994), banded cucumber beetle (Huang et al., 2002), and two lepidopterans, *Trichoplusia ni* (Hübner) and *Spodoptera exigua* (Hübner) (both Lepidoptera: Noctuidae) (Sethi et al., 2006). These studies suggested that Valmaine lacks feeding stimulants or contains feeding deterrents, either in the leaf cuticle or the leaf interior. Huang et al. (2003a) reported that leaf surface chemicals were not responsible for resistance in Valmaine and suggested that chemicals inside the leaf may play a role. However, incorporation of freeze-dried leaves of Valmaine into an artificial diet did not deter feeding by *D. balteata* adults and neither did application of Valmaine latex on the leaf surface of a favorite food, lima bean (Huang et al., 2003b). It is possible that the activity of physical and/or chemical defenses in latex or leaf tissue may have been reduced or eliminated when whole leaves were dried and powdered. Furthermore, the physical and chemical properties of latex may have changed when applied on lima bean leaves due to drying of the latex and/or oxidation of chemical constituents.

In free-choice situations, *L. trifolii*, *D. balteata*, *T. ni*, and *S. exigua* preferred to feed on mature leaves of Valmaine over young or middle-aged leaves (Nuessly & Nagata, 1994; Huang et al., 2002; Sethi et al., 2006). The avoidance of young and middle-aged leaves of Valmaine may have been due to the presence of high amounts of latex and/or the chemical constituents of latex; latex from young and middle-

aged leaves is pure white and viscous, whereas latex from mature leaves is watery and translucent (Sethi et al., 2006).

In this study, we report on the possible deterrent role of latex against beetle feeding on artificial diet treated with freshly extracted latex from either Valmaine or Tall Guzmaine under choice and no-choice conditions. Additional tests were conducted using latex extracted from young vs. mature leaves of these two cultivars to study the role of leaf age in the expression of latex deterrence. Lastly, samples of supernatant material collected following dissolution of latex from both cultivars in water/methanol combinations or methylene chloride and centrifugation were applied to diet disks under no-choice situations to determine whether differences in latex chemistry between Valmaine and Tall Guzmaine contribute to the multiple insect resistance observed in Valmaine.

Materials and methods

Plants and insects

Valmaine and Tall Guzmaine seeds were germinated in the laboratory by placing them overnight in a Petri dish lined with a wet filter paper. Germinated seeds were planted in a transplant tray filled with commercial soil mix (MetroMix 200; Sun Gro Horticulture, Bellevue, WA, USA) in a greenhouse with natural light at a mean temperature of 27 °C (24–32 °C) and a mean relative air humidity of 68% (44–94% r.h.). After 2 weeks, seedlings were transplanted into 15-cm plastic pots filled with MetroMix 200. Plants were irrigated daily and fertilized once a week with 10 ml of a 10 g/l solution of soluble fertilizer (Peters 20-20-20, N-P-K; W.R. Grace, Fogelsville, PA, USA).

Bush lima bean [*Phaseolus lunatus* L. (Fabaceae)] cultivar Fordhook 242 (Illinois Foundation Seeds, Champagne, IL, USA) were planted in a transplant tray filled with MetroMix 200. Lima bean plants were irrigated daily and fertilized once a week after the first true leaf stage with the same solution used for lettuce plants.

A colony of *D. balteata* was established in 2003 from a wild population of adults collected from spiny amaranth, *Amaranthus spinosus* L. (Amaranthaceae), and primrose willow, *Ludwigia peruviana* (L.) H.Hara (Onagraceae), in Belle Glade, FL, USA. The colony was supplemented with wild individuals to increase genetic diversity in 2005 and 2006. Adults of *D. balteata* were fed on lima bean leaves and sweet potato tubers, and larvae were reared on corn seedling roots as per Huang et al. (2002).

Artificial diet preparation

Dry mix for artificial diet is commercially available and has been shown to support the adult stage of *D. balteata* (Creighton & Cuthbert, 1968). All materials required for

preparing and dispensing the diet were thoroughly sanitized with sodium hypochlorite solution (Clorox, Oakland, CA, USA) diluted 1:5 with deionized water. A 100-ml quantity of southern corn rootworm artificial diet (Bio-Serv, Frenchtown, NJ, USA) was prepared as follows. Sterile deionized water (100 ml) and agar (1.74 g) were heated on a hot plate to boiling. Once the agar had cooled to approximately 40 °C, KOH solution (1 ml) and diet dry mix (14.91 g) were added to it and thoroughly mixed to avoid the formation of lumps. The liquid diet was dispensed into two glass Petri dishes (9 cm in diameter). The diet was allowed to cool before the Petri dishes were covered with glass lids. The Petri dishes were wrapped completely in plastic wrap and aluminium foil, and stored in a refrigerator (4–6 °C) for up to 3 h.

Latex collection and solvent extraction

In a pilot study, we incorporated fresh latex directly into the artificial diet for *D. balteata* adults at two concentrations (0.1 and 0.2%) and recorded diet consumption by *D. balteata* adults. Latex did not deter feeding of *D. balteata* adults when presented in this manner. Therefore, in this study, we applied freshly extracted latex from either Valmaine or Tall Guzmaine to the entire surface of disks made from artificial diet and confined *D. balteata* adults under choice and no-choice conditions. We chose artificial diet as a substrate for application of the latex, rather than lima bean leaves used by Huang et al. (2003b), because latex remained moist longer on diet than on lima bean leaves. In addition, latex-treated diet disks facilitated the direct exposure of *D. balteata* adults to latex. As the diet disks were totally covered with latex on all sides, it somewhat simulated the natural situation where an insect chewing a lettuce plant is directly exposed to latex.

Latex (70 μl) was collected from the bases (where leaf lamina joins the stem) of young and middle-aged leaves of individual plants (9–10 true leaf stage), sites where there was a rapid exudation of latex upon cutting. The cuts were made using a disposable scalpel blade (Feather, Osaka, Japan). The latex was collected using a silanized 100-μl glass capillary tube inserted into a microdispenser (Drummond Scientific Company, Broomall, PA, USA) 60 s after the leaf base was cut. A cork borer (1.5 cm in diameter) was used to punch out disks (1 cm thick) from cooled artificial diet. Latex (70 μl) from an individual plant was applied, immediately after collection, onto the top surface and sides of a diet disk using a microdispenser.

Four solvent combinations, that is, water, water:methanol (20:80), water:methanol (50:50), and methylene chloride, were used to extract chemical constituents of latex. Latex (70 μl) was collected from an individual plant in the same way as described above and immediately dissolved in 10 times volume of the solvent. After dissolution, samples were centrifuged at 1 200 ***g*** for 20 min and supernatant was collected. The supernatant was reduced down to 1/10th of its volume by evaporating with nitrogen gas. An amount of extract, equivalent to 70 μl latex, was applied to each diet disk for use in the following bioassays.

Bioassay conditions

For all experiments described below, an experimental unit consisted of two diet disks and three pairs of unfed *D. balteata* adults within a plastic ventilated container (10 × 10 × 8 cm). Unfed adults that had emerged within 48 h of the start of the experiment were used in all tests. The diet disks were placed on the bottom of the container and beetles were allowed to feed on the diet for 16 h. Each experimental unit was replicated 15 times. The experiments were carried out at 25 ± 1 °C in a laboratory at a photoperiod of L14:D10. In all tests, the number of adults feeding on each diet disk was recorded at 15, 30, 60, and 90 min after their release into the bioassay chambers.

Choice tests and no-choice tests with fresh latex

Choice tests were conducted to determine whether *D. balteata* adults showed a preference between diet disks treated with latex from Valmaine or Tall Guzmaine. Three treatment combinations were studied: latex from Valmaine vs. latex from Tall Guzmaine, latex from Valmaine vs. control (diet disk without latex application), and latex from Tall Guzmaine vs. control. Three treatments (latex from Valmaine, latex from Tall Guzmaine, and control) were studied in a no-choice situation, each experimental unit containing two disks of the same treatment.

Dry weight of diet consumed in a 16-h period was calculated for comparison among the treatments. To determine dry weight, an additional 10 diet disks from each treatment (Valmaine latex-treated, Tall Guzmaine latex-treated, and control) were weighed individually (disk fresh weight) before they were put into an oven at 50 ± 5 °C. After 3 days, these diet disks were reweighed individually (disk dry weight). A dry/fresh weight ratio was calculated for each diet disk and averaged over the 10 disks. The diet disk from each treatment was weighed prior to the start of the experiment, and dry weight was computed by multiplying with the corresponding average dry/fresh weight ratio. After 16 h of exposure to beetle feeding, the diet disk was dried in the oven for 3 days at the same temperature. The dry weight of diet consumed was calculated as the difference between initial and final dry weights.

Choice tests using latex from young and mature leaves

Choice tests were conducted to determine whether *D. balteata* adults exhibit any preference between diet disks treated

with latex from young or mature leaves of each cultivar Valmaine and Tall Guzmaine, separately. Two treatment combinations were studied: latex from young leaves vs. latex from mature leaves of Valmaine, and latex from young leaves vs. latex from mature leaves of Tall Guzmaine. The dry weight consumption of young and mature latex-treated diets of each cultivar in 16 h was recorded as described above. Total diet consumed per three pairs of adults for 16 h was calculated by adding consumption of the two diet disks in each container in each treatment.

No-choice tests using latex extracts

Fifteen treatments were studied: five for Valmaine – latex dissolved in water, water:methanol (20:80 vol/vol), water:methanol (50:50), methylene chloride, and fresh latex without solvent; five for Tall Guzmaine – latex dissolved in water, water:methanol (20:80), water:methanol (50:50), methylene chloride, and fresh latex without solvent; and five for control – the four solvent combinations without latex and untreated diet. Each experimental unit contained two disks of the same treatment. The dry weights of Valmaine- and Tall Guzmaine-extract treated and control diets disks consumed in 16 h were calculated as above.

Statistical analysis

For all choice and no-choice tests using latex, the number of adults feeding on diet 15, 30, 60, and 90 min after release was analyzed as a repeated measures design using Proc GLIMMIX (SAS Institute, 2003). In each choice test (Valmaine vs. Tall Guzmaine, Valmaine vs. control, and Tall Guzmaine vs. control), data on number of adults feeding were analyzed separately as a 2 × 4 factorial design, in which latex was treated as one factor with two levels, and time interval after beetle release was treated as the other factor with four levels (15, 30, 60, and 90 min). In no-choice tests, data on number of adults feeding were analyzed as a 3 × 4 factorial design, in which latex was treated as one factor with three levels (Valmaine, Tall Guzmaine, and control), and time interval after beetle release was treated as the other factor with four levels. Both variables (latex and time interval) were fixed. Fifteen groups of six beetles (i.e., replications) were randomly assigned to each level of latex, meaning that beetles were nested within latex levels. Each group of six beetles was tested four times (levels of time interval).

In no-choice tests using latex extracts, the data on number of beetles feeding were analyzed separately using Proc GLM (SAS Institute, 2003) at each time interval with latex extract as a fixed effect and replications as a random effect. The dry weights of Valmaine, Tall Guzmaine, and control diets consumed in 16 h were analyzed by paired t-tests using Proc MEANS (SAS Institute, 2003) for all choice tests and by analysis of variance (ANOVA) using Proc GLM with latex as a fixed effect and replications as a random effect for all no-choice tests. The total dry weight consumed, calculated by adding consumption of the two diet disks in each choice test using latex from young and mature leaves, including control disks, was also analyzed by ANOVA using Proc GLM with latex as a fixed effect and replications as a random effect. Tukey's honestly significant difference (HSD) test with a significance level of α = 0.05 (SAS Institute, 2003) was used for post-hoc means separation.

Deterrence coefficients (relative and absolute) (Nawrot et al., 1986) were calculated based on the amount of diet consumed. All the data from choice and no-choice tests were pooled and used to determine coefficients using the following equations:

Relative (R) = [(C − T)/(C + T)] × 100 (choice test) and

Absolute (A) = [(CC − TT)/(CC + TT)] × 100 (no-choice test),

where C and CC are the consumption of control diet (without latex), and T and TT are the consumption of latex-treated diet (Valmaine or Tall Guzmaine) in choice and no-choice tests, respectively. The deterrent activity of the latex-treated diets was expressed by the total coefficient of deterrence (D = A + R). The deterrence coefficients were analyzed by two-sample t-tests using Proc TTEST (SAS Institute, 2003).

Results

Latex choice and no-choice tests

Latex treatment had a significant effect on the number of insects feeding in all three choice tests: Valmaine vs. Tall Guzmaine ($F_{1,28}$ = 64.83, P = 0.0001), Valmaine vs. control ($F_{1,28}$ = 99.27, P = 0.0001), and Tall Guzmaine vs. control ($F_{1,28}$ = 5.68, P = 0.0241). Beetles avoided feeding on diet treated with Valmaine latex (Figure 1A,B). The number of insects feeding on diet treated with Valmaine latex was negligible compared to the number feeding on diet treated with Tall Guzmaine latex (Figure 1A) and control diet (Figure 1B). The number of insects feeding increased over time (i.e., 15, 30, 60, and 90 min) in all choice tests (Valmaine vs. Tall Guzmaine: $F_{3,84}$ = 7.28, P = 0.0002; Tall Guzmaine vs. control: $F_{3,84}$ = 9.83, P = 0.0001; and Valmaine vs. control: $F_{3,84}$ = 24.87, P = 0.0002) (Figure 1). Significant interactions were found between latex treatment and time interval in the choice tests involving Valmaine and Tall Guzmaine ($F_{3,84}$ = 8.56, P = 0.0001) and Valmaine and the control diet ($F_{3,84}$ = 28.47, P = 0.0001). In contrast, there was no significant interaction found in the choice test between Tall Guzmaine latex-treated diet disks and control disks ($F_{3,84}$ = 1.44, P = 0.2374) (Figure 1C). Beetles
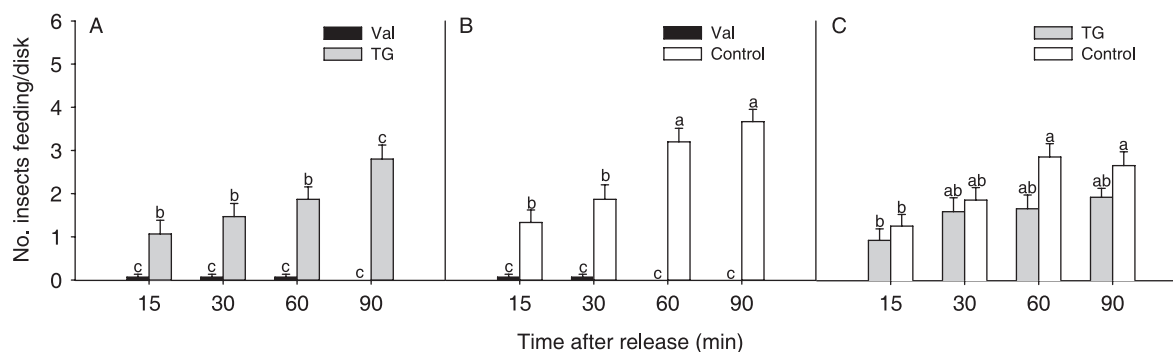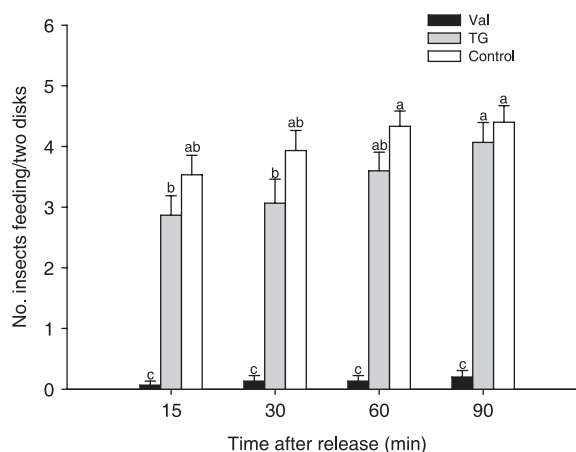
Figure 1 Mean (+ SEM) number of *Diabrotica balteata* adults feeding on artificial diet disks treated with (A) latex from resistant Valmaine (Val) vs. latex from susceptible Tall Guzmaine (TG), (B) latex from Valmaine vs. control (diet disk without latex application), and (C) latex from Tall Guzmaine vs. control in choice tests. Bars capped with different letters within the same panel differ significantly (Tukey's HSD test: P≤0.05).

consumed significantly less diet treated with Valmaine latex (Table 1). They ate 2.9 times more on Tall Guzmaine latex-treated diet than diet treated with Valmaine latex in a choice between Valmaine and Tall Guzmaine. Furthermore, beetles also consumed 4.5 times more control diet than diet treated with Valmaine latex in a choice between Valmaine and control. They consumed 1.5 times less diet treated with Tall Guzmaine than control diet.

In no-choice tests, latex also had a significant effect on the number of insects feeding on diets ($F_{2,42} = 109.46$, P = 0.0001). Significantly fewer insects fed on diet treated with Valmaine latex than on Tall Guzmaine latex-treated and control diets (Figure 2). No significant interaction was found between latex treatment and time interval

($F_{3,26} = 1.74$, P = 0.1179). Beetles consumed 4.7 and 6.3 times more Tall Guzmaine latex-treated and control diets, respectively, than Valmaine latex-treated disks ($F_{2,42} = 168.31$, P = 0.0001) (Table 1).

Figure 2 Mean (+ SEM) number of *Diabrotica balteata* adults feeding on two artificial diet disks treated with latex from resistant Valmaine (Val), disks treated with latex from susceptible Tall Guzmaine (TG), and control diet disks in no-choice tests. Bars capped with different letters differ significantly (Tukey's HSD test: P≤0.05).

Valmaine latex exhibited strong deterrence against beetles in both choice and no-choice bioassays (Table 2). Both relative and absolute coefficients of deterrence for Valmaine latex-treated diets were significantly higher than those for Tall Guzmaine latex-treated diets. The total coefficient of deterrence of Valmaine latex was 3.9 times higher than that of Tall Guzmaine latex.

Table 1 Dry weight consumption of diet disks treated with Valmaine (Val) or Tall Guzmaine (TG) latex under choice and no-choice tests by six *Diabrotica balteata* adults in 16 h

| | Mean diet consumed ± SEM (mg) | | | |
| | Treatment | | | |
Tests	Val latex	TG latex	Control	P-value[1]
Choice				
Val vs. TG	5.4 ± 0.5	15.5 ± 0.7	–	0.0001
Val vs. control	5.5 ± 0.5	–	24.7 ± 0.5	0.0001
TG vs. control	–	14.4 ± 0.5	21.9 ± 0.6	0.0001
No-choice	7.3 ± 0.4c	34.6 ± 1.1b	46.2 ± 2.4a	0.0001

[1]P-value from paired t-test in case of choice tests.
Means ± SEM followed by different letters in no-choice test differed significantly (P≤0.05) using ANOVA ($F_{2,42} = 168.31$, P = 0.0001) and Tukey's HSD test.

Table 2 Feeding deterrent activity of latex against *Diabrotica balteata* adults when artificial diet disks were treated with latex from either resistant Valmaine (Val) or susceptible Tall Guzmaine (TG) in choice and no-choice tests

Latex	Deterrence coefficients		
	Relative	Absolute	Total
Val	63.6	72.7	136.3
TG	20.7	14.4	35.0
P-value	0.0001	0.0001	0.0001

P-value from two-sample t-test.

Choice tests using latex from young and mature leaves

In the choice test involving latex from Valmaine, latex significantly affected the number of insects feeding on diet ($F_{1,28}$ = 61.87, P = 0.0001), but insect feeding was not significantly affected by latex treatment in Tall Guzmaine choice tests ($F_{1,28}$ = 1.84, P = 0.81). Significantly fewer insects fed on diet treated with latex from young leaves than on diet treated with latex from mature leaves of Valmaine (Figure 3). Adult preference for diet treated with latex from mature leaves of Valmaine increased significantly with time ($F_{3,84}$ = 30.95, P = 0.0001). In the Tall Guzmaine latex choice test, the number of beetles feeding on diet disks treated with latex from young leaves did not differ significantly from that on disks treated with latex from mature leaves (Figure 3). The number of beetles feeding on both Tall Guzmaine diets increased significantly with time ($F_{3,84}$ = 39.44, P = 0.0001).

Beetles consumed 7.2 times more diet treated with latex from mature Valmaine leaves than treated with latex from young Valmaine leaves (Table 3). Diet consumption did not differ significantly between diet disks treated with latex from young and mature leaves of Tall Guzmaine. The total diet consumed in the Valmaine latex choice test (sum of the consumption on the two disks) did not differ significantly from the amount eaten in the Tall Guzmaine latex choice test, but was significantly less than the amount consumed in the control diet test.

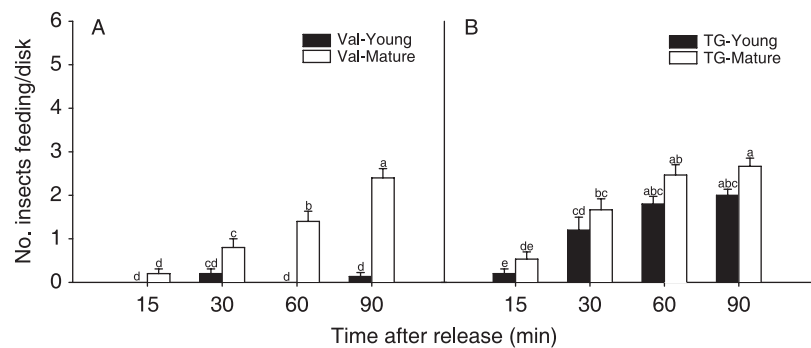**No-choice tests using latex extracts**

Water extracts of both Valmaine and Tall Guzmaine were yellow in color, but the color of the Valmaine extract was more intense than that of the Tall Guzmaine extract. Water:methanol (20:80) extracts of both cultivars were colorless. The water:methanol (50:50) extract of Tall Guzmaine was colorless, but it was yellow in the case of Valmaine. Methylene chloride extracts of both cultivars were white in color and sticky.

Treatment with latex extracts had a significant effect on the number of insects feeding on diet after 15 min ($F_{14,196}$ = 11.97, P = 0.0001), 30 min ($F_{14,196}$ = 12.60, P = 0.0001), 60 min ($F_{14,196}$ = 24.42, P = 0.0001), and 90 min of release ($F_{14,196}$ = 31.93, P = 0.0001). Significantly fewer insects fed on diet disks treated with a water:methanol (20:80) extract of Valmaine latex than on diets treated with all other Valmaine and Tall Guzmaine latex extracts, as well as all the control diets (Figure 4). In addition, diet consumption was also significantly affected by the latex extract treatment ($F_{14,196}$ = 95.01, P = 0.0001). Beetles consumed significantly less diet treated with water:methanol (20:80) extract of Valmaine latex than diet treated with any other latex extract or control diet (Figure 5). The number of insects feeding on disks (Figure 4) and amount consumed (Figure 5) on diet disks treated with the water:methanol (20:80) extract of Valmaine latex were equivalent to those values for diet treated with fresh Valmaine latex.

Discussion

Evidence presented here indicates that resistance against *D. balteata* found in the romaine lettuce cultivar, Valmaine, is due to latex. Adult *D. balteata* were found more frequently on diets treated with latex from Tall Guzmaine than on diets treated with Valmaine latex in both choice

Figure 3 Mean (+ SEM) number of *Diabrotica balteata* adults feeding on artificial diet disks treated with latex from young or mature leaves of (A) resistant Valmaine (Val) and (B) susceptible Tall Guzmaine (TG) in choice tests. Bars capped with different letters within a panel differ significantly (Tukey's HSD test: P≤0.05).
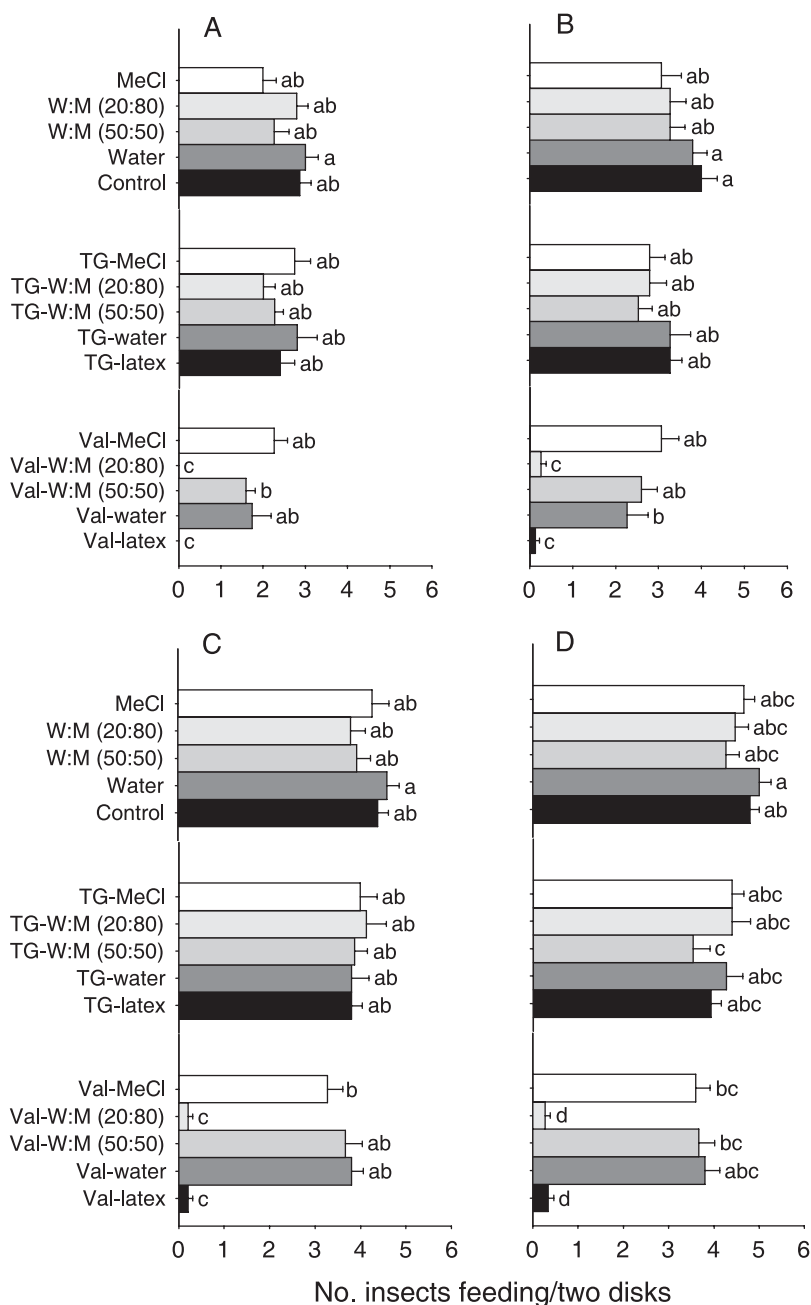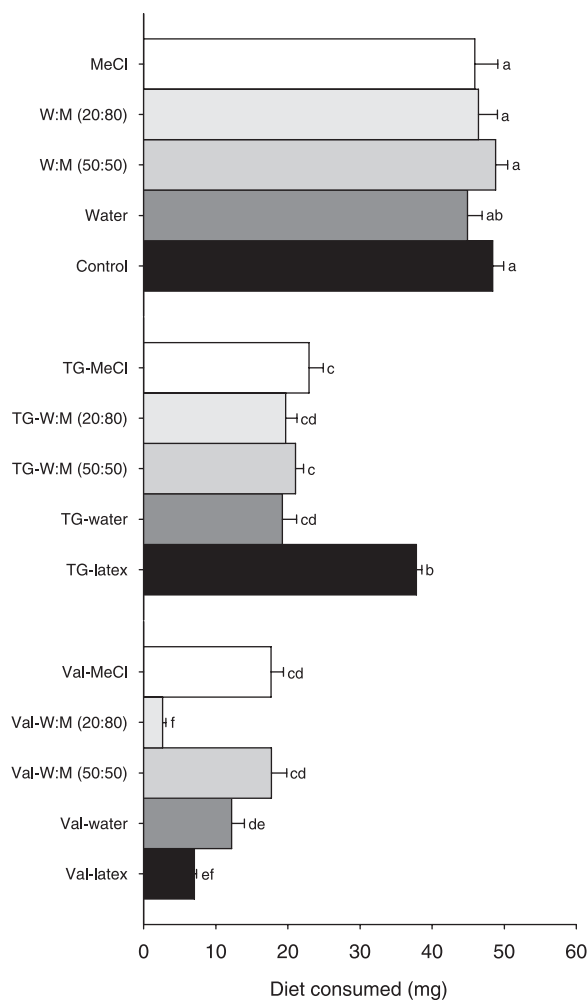
Figure 4 Mean (+ SEM) number of *Diabrotica balteata* adults feeding on two artificial diet disks treated with latex extracts from resistant Valmaine (Val), susceptible Tall Guzmaine (TG), and controls in no-choice test at (A) 15 min, (B) 30 min, (C) 60 min, and (D) 90 min after release. Bars capped with different letters within the same panel differ significantly (Tukey's HSD test: P≤0.05). W, water; M, methanol; and MeCl, methylene chloride.

and no-choice tests. In latex choice tests, beetles flew immediately to the roof and walls of the container whenever they approached the Valmaine latex-treated diet disk, whereas the beetles started feeding on the Tall Guzmaine latex-treated diet disk whenever they approached it. In latex no-choice tests, beetles generally returned to the roof and walls of the container after approaching the Valmaine latex-treated diet disks several times. The behavior of the beetles on diet treated with water:methanol

(20:80) extracts of Valmaine latex was similar to that for diet treated with pure Valmaine latex. Before biting the latex-treated diet disk, beetles inspected it at a close range, antennating and palpating it. In cases where the beetles landed directly on the disk, they appeared to sense the deterrent with their tarsi, even before antennating and palpating the disk, and flew back to the container walls immediately. Beetles performed frequent and more vigorous grooming of antennae and tarsi by passing them

Table 3 Dry weight of diet consumed by six *Diabrotica balteata* adults in 16 h when given a choice between diet disks treated with latex from either young or mature leaves of resistant Valmaine or susceptible Tall Guzmaine lettuce cultivars

Cultivar	Choice	Diet consumed (mg)[2]	Total diet consumed (mg)[3]
Valmaine	Young latex-treated diet vs.	3.7 ± 0.6b	29.8 ± 2.2b
	Mature latex-treated diet	26.1 ± 1.9a	
Tall Guzmaine	Young latex-treated diet vs.	18.1 ± 2.0a	38.7 ± 3.9ab
	Mature latex-treated diet	20.5 ± 2.2a	
Control[1]	–	–	50.1 ± 5.2a

[1]Both disks were untreated in control diet.

[2]Means ± SEM followed by different letters within cultivar differed significantly using paired t-test.

[3]Means ± SEM followed by different letters differed significantly (P≤0.05) using ANOVA ($F_{2,42} = 6.69$, P = 0.0030) and Tukey's HSD test.

Figure 5 Dry weight (mean + SEM) of diet consumed by six *Diabrotica balteata* adults in 16 h, when both diet disks were treated with Valmaine (Val) or Tall Guzmaine (TG) latex extracts under no-choice situations. Bars capped with different letters differ significantly (Tukey's HSD test: P≤0.05). W, water; M, methanol; and MeCl, methylene chloride.

through mouthparts after contact with Valmaine latex compared to Tall Guzmaine latex. Further tarsal grooming was also done by scraping the legs on the elytra. The alighting behavior of the beetles observed in our choice and no-choice tests suggests that contact chemosensilla are present on their antennae, palps, and tarsi (Chapman, 2003). These types of chemosensilla have been reported in other chrysomelids, such as on the antennae of the cabbage stem flea beetle, *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae) (Isidoro et al., 1998), maxillary appendages of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (Chyb et al., 1995; Eichenseer & Mullin, 1996), and tarsomeres of the Klamath beetle, *Chrysolina brunsvicensis* Gravenhorst (Coleoptera: Chrysomelidae) (Rees, 1969), and have been found to discriminate between phagostimulants and phagodeterrents. Antennal and tarsal grooming, similar to that observed by us with *D. balteata*, has been reported in the crucifer flea beetle, *Phyllotreta cruciferae* Goeze (Coleoptera: Chrysomelidae), as an important part of the pre-feeding behavior for recognizing host and non-host crucifers (Henderson et al., 2004).

Adult *D. balteata* consumed significantly less Valmaine latex-treated diet compared to Tall Guzmaine latex-treated diet in both choice and no-choice tests. Huang et al. (2003b) reported that latex from both Valmaine and Tall Guzmaine was very deterrent to beetle feeding when applied on lima bean leaves. We believe that Tall Guzmaine latex in the studies of Huang et al. (2003b) showed very high deterrence due to changes in its chemical properties (possibly oxidation) after drying on the lima bean leaf surface. In our studies, the precisely defined quantities of latex (70 µl) applied to diet disks did not dry significantly within the 16-h exposure period to beetles due to moisture from the artificial diet, which perhaps prevented changes in the chemical properties of the latex. Both cultivars produce latex upon wounding, but the much higher coefficient of

deterrence for Valmaine latex compared to Tall Guzmaine latex observed in our study argues that Valmaine latex is more deterrent than Tall Guzmaine due to its physical or/and chemical properties. Furthermore, we observed that beetles on Valmaine moved away from an initiated feeding site very quickly after coming in contact with the exuded latex and started test bites much farther away from the previous bites than they did on Tall Guzmaine (A Sethi, unpubl). These properties may be due to the original chemicals produced by the plants or new chemicals produced by the action of certain plant enzymes, such as phenylalanine ammonia lyase, polyphenol oxidase, and peroxidase. Valmaine also partially or totally lost its resistance in feeding bioassays using detached leaves or leaf disks, where latex exudation was greatly reduced (Huang et al., 2003c). This observation further supports the defensive role of latex in resistant Valmaine.

The strong deterrent activity of Valmaine latex extracted with water:methanol (20:80) provides compelling evidence for the chemical basis of resistance in Valmaine against *D. balteata*. The ability of water:methanol (20:80) to extract deterrent constituents from Valmaine latex suggests that moderately polar compounds in Valmaine latex account for its feeding deterrence. The defensive role of latex due to chemical constituents against insects has been reported in many plant systems, such as milkweed (Dussourd & Hoyle, 2000), mulberry (Konno et al., 2006), papaya (Konno et al., 2004), and chicory (Rees & Harborne, 1985). Various organic compounds, such as phenolics and terpenoids, have been reported in latex of *Lactuca* spp. (Crosby, 1963; Gonzalez, 1977; Cole, 1984; Sessa et al., 2000), and their defensive role as phytoalexins has been reported against plant diseases (Bennett et al., 1994; Bestwick et al., 1995).

Latex from young leaves of Valmaine strongly deterred the feeding of *D. balteata* adults in a choice between diets treated with latex from young and mature leaves. Sethi et al. (2006) found that latex from young and mature leaves differed in terms of milkiness and viscosity. Such differences in milkiness arise due to differences in the refractive indices of the dispersing particles (mainly terpenoids) and the dispersing medium (Esau, 1965; Fahn, 1990). Thus, latex from young leaves may be richer in dispersing particles, and the relatively higher amount of dispersing particles may have a specific purpose related to plant defense. Young leaves are typically better defended than mature leaves due to the presence of higher quantities of latex and its associated chemical components (Swain, 1977; Spilatro & Mahlberg, 1986). In the chicory plant, *Cichorium intybus* L. (Asteraceae), sesquiterpene lactones were present in the highest amounts in the most actively growing regions of the plant and possessed antifeedant properties against *Schistocerca gregaria* Forskål (Orthoptera:

Acrididae) (Rees & Harborne, 1985). Young vines of sweet-potato, *Ipomoea batatas* (L.) Lam. (Convolvulaceae), possessed more latex and exhibited less damage due to the sweetpotato weevil, *Cylas formicarius* (F.) (Coleoptera: Curculionidae), than mature vines (Data et al., 1996). Latex exudation is higher in growing regions than in mature regions of great bindweed, *Calystegia silvatica* (Kit.) Griesb (Convolvulaceae) (Condon & Fineran, 1989).

The anatomy of laticifers changes during the course of their ontogeny (Olson et al., 1969). The number of laticifers and their contents decrease with increasing proximity to roots (Condon & Fineran, 1989; Monacelli et al., 2005). In mature leaves, the protoplast of laticifers degenerates near senescence, indicating a low level of metabolism (Fineran, 1982, 1983). Fusion of latex particles has also been seen in mature leaves when much of the latex has already vanished (Dickenson, 1963; Heinrich, 1967; Fineran, 1982). Plug-like masses of callose have been reported at the bases of mature leaves and no latex at all or much reduced amounts of latex exude when such leaves are severed, completely or partially, from the plant (Spencer, 1939). Young leaves have discrete files of laticifers separated by end walls, while laticifers differentiate by breakdown of end walls in mature leaves (Condon & Fineran, 1989). Thus, laticifers of young leaves may have more turgor pressure resulting in profuse latex exudation compared to mature leaves, making it more likely that insect mandibles will be exposed to latex during test bites on intact leaves.

Our data support the hypothesis that latex has a definite role in the expression of resistance in Valmaine lettuce to *D. balteata*, and differences in latex chemistry between the two cultivars may account for this. Future research on the isolation of latex constituents and their biological activity is required to better understand the mechanism of resistance in Valmaine lettuce. This knowledge may contribute to the development of new cultivars expressing insect resistance along with superior horticultural traits through conventional and transgenic breeding approaches.

Acknowledgements

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